DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 3/30/09 has been entered.

Applicant's arguments filed 3/30/09 have been fully considered. Claims 45-46, 50-54, 60-61, 63-65, 68-71, 87-89, 91 are pending. Claims 63, 91 are withdrawn to a non-elected invention. Claims 1-44, 47-49, 55-59, 62, 66-67, 72-86, 90, 92-132 are canceled.

Claims 45-46, 50-54, 60-61, 64-65, 68-71, 87-89 are under consideration.

For clarity of the record as discussed in the interview summary claims 63, 91 are withdrawn to a non-elected invention (see response to election restriction requirement filed on 2/12/07). The claims in the previous office action were examined based on the claim 45 interpretation that the culture of hES cells is in the presence of an embryonic cell, wherein said embryonic cell broadly embraces any embryonic cell including a vascular endothelial cell. Applicant's representative on the phone interview 5/5/09 clarified the record and proposed an amendment that claim 45 embraces "wherein said embryonic cell is an endodermal or ectodermal cell and not a vascular endothelial cell". Applicant's representative agreed she will revisit the response to the restriction requirement filed on 2/12/07 to concur with the Examiner that the election of the hEs cell is to a cardiomyocyte and not to a vascular endothelial cell as claimed in the current amendment. Therefore, the claims are examined to the extent the hES cell is a cardiomyocyte and not a vascular endothelial cell and second the hES cell is cultured in the presence of an endodermal or ectodermal cell.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims **45-46, 63, 64, 71, 87, 88, 89** rejection under 35 U.S.C. 102(b) as being anticipated by Itskovitz-Eldor et al, (Molecular Medicine, 6(2): 88-95, 2000) is withdrawn in view of the amendment.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 45-46, 50-54, 61, 63-65, 68-71, 87-89, 91 rejection under 35 U.S.C. 103(a) as being unpatentable over Itskovitz-Eldor et al, (Molecular Medicine, 6(2): 88-95, 2000) in view of Sugi et al, (Developmental Dynamics, 200: 155-162, 1994); Zhu et al, (Developmental Dynamics, 207: 429-438, 1996); Lough et al, (Developmental Dynamics, 217: 327-342, 2000); Klug et al, (J Clin Invest, 98: 216-224, 1996) is withdrawn in view of the amendment.

Claims 45-46, 50-54, 60, 64-65, 68-71, 87-89 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Reubinoff et al** (Nature Biotechnology, 18: 399-404, 2000) in view of **van den Eijnden-van Raaij et al,** (Mechanisms of Development, 33: 157-166, 1991 (IDS));

Skerjanc, (Trends Cardiovasc Med 1999;9:139–143, 1999); **Itskovitz-Eldor et al**, (WO 00/70021).

Reubinoff et al (Nature Biotechnology, 18: 399-404, 2000) teaches a method for the derivation of cardiomyocytes from the hES2 cells (p 399-400). Beating cardiomyocytes were observed following co-culturing of the hES2 cells with mouse embryonic fibroblasts (MEFs) (p 401, 2nd column, 2nd paragraph). Reubinoff differs from the present invention for not teaching co-culture with endothelial cells (END2).

However at the time of the instant invention **van den Eijnden-van Raaij et al,** (Mechanisms of Development, 33: 157-166, 1991) teaches earlier co-culture of END2 cells with mouse P19 embryonal carcinoma (EC) cells, a mouse embryonal carcinoma cell line with pluripotent differentiation properties, and with mESC had shown the beating areas appeared in the aggregated cells (figure 1, p 158). **van den Eijnden-van Raaij et al,** teach addition of conditioned medium from the END2 cells into the ES cells onto culture plastic dishes where they could not attach did not result in the differentiation of the ES cells (p 159, 1st column). **Skerjanc,** (Trends Cardiovasc Med 1999;9:139–143, 1999) teaches P19 cells provide a useful system for examining mechanisms of cardiac and skeletal muscle development. Many studies have indicated that the mechanisms involved in P19 cell differentiation emulate those observed in early stages of embryonic development. Significantly, the temporal pattern of expression of factors involved in mesoderm induction and commitment to either cardiac or skeletal muscle lineages corresponds to that in the embryo (Table 1). Furthermore, overexpression of MyoD, myogenin, GATA-4, Nkx2-5, and MEF2C led to activation of endogenous gene expression that was consistent with the known function of each of these factors (Table 2). In every example of overexpression, cellular aggregation resulting in mesoderm induction was required

to activate the function of the transfected gene. Skerjanc suggests the P19 system offers certain technical advantages for biochemically examining the network of signals regulating mesoderm induction and commitment to either the cardiac or skeletal muscle lineages or P19-derived myocytes appear to recapitulate properties of mammalian myocytes. **Itskovitz-Eldor et al**, (WO 00/70021) teaches a method for providing a) human embryonic stem (hES) cells; b) growing the hES cells in vitro in a vessel under conditions in which the cells undergo differentiation and the cells or aggregates thereof do not adhere to the vessel wall; and c) formation of EBs from said cells. Itskovitz-Eldor et al, also teaches the hES cells (hEBs) comprise mesoderm, ectoderm and endoderm lineage cells (see claims 1-8). Thus, Itskovitz-Eldor et al, taught a method for population of hES cells including cells of different lineages such as endothelial cells structurally the same as embraced by instant invention method.

Accordingly, in view of the teachings of Reubinof/van den Eijnden-van Raaij/ Skerjanc,/ Itskovitz-Eldor it would have been obvious for one of ordinary of skill in the art, at the time of the instant invention to combine the method of Reubinof/van den Eijnden-van Raaij/ Skerjanc,/ Itskovitz-Eldor regarding inducing differentiation of an undifferentiated hES cell into mesodermal cell as taught by the combined cited references with a reasonable expectation of success in a co-cultures system of hES cells and endothelial cells. It is noted that all the claimed elements were known in the art and one of skill in the art could have combined the elements as claimed by known method with no change in their respective function and the combination would have yielded nothing more than predictable results. In the instant case, Reubinoff/van den Eijnden-van Raaij teach a method for co-culturing mES cells with endothelial cells and Skerjanc,/Itskovitz-Eldor provide motivation of using human ES cells by disclosing progress made in treatment and prevention of human diseases by applying the human ES cells differentiated into cardiomyocytes by applying to human ES cells using directed differentiation

by endothelial cells. One of ordinary of skill in the art would have been motivated to obtain hES cells by the co-culture of endothelial cells disclosed by Reubinoff/Itskovitz-Eldor in transplant studies. Given Skerjanc suggests the P19 system offers certain technical advantages for biochemically examining the network of signals regulating mesoderm induction and commitment to either the cardiac or skeletal muscle lineages and P19-derived myocytes appear to recapitulate properties of mammalian myocytes and the claims embrace a mixing of hES cells with endothelial cells of any percentage as taught by Reubinoff/Itskovitz-Eldor one who would practice the invention would have had a reasonable expectation of success because the combined cited references embrace the potential of co-culturing hES cells with endothelial cells for transplantation therapy.

Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

Claim **61** is rejected under rejected under 35 U.S.C. 103(a) as being unpatentable over Reubinoff et al (Nature Biotechnology, 18: 399-404, 2000) in view of van den Eijnden-van Raaij et al, (Mechanisms of Development, 33: 157-166, 1991); Skerjanc, (Trends Cardiovasc Med 1999;9:139–143, 1999); Itskovitz-Eldor et al, (WO 00/70021) and further in view of **Carpenter et al** (US 20020081724 A1).

The teachings of Reubinof/van den Eijnden-van Raaij/ Skerjanc,/ Itskovitz-Eldor apply here as stated above.

Reubinof/van den Eijnden-van Raaij/ Skerjanc,/ Itskovitz-Eldor do not teach the hES cells separated by a filter or cellular matrix.

However at the time of the instant invention Carpenter teaches that it is routine in the art of co-culturing stem cells to use matrigel as a separation matrix [0093-0095]. Particularly suitable as a substrate for feeder-free pPS culture are extracellular matrix components (derived

from basement membrane, or forming part of adhesion molecule receptor-ligand couplings). A commercial preparation is available from Becton Dickenson under the name Matrigel.RTM., and can be obtained in regular or Growth Factor Reduced formulation. Both formulations are effective. Matrigel.RTM. is a soluble preparation from Engelbreth-Holm-Swarm tumor cells that gels at room temperature to form a reconstituted basement membrane.

Accordingly, in view of the teachings of **Carpenter et al** it would have been obvious for one who would practice the invention would have had a reasonable expectation of success because the combined cited references embrace the potential of co-culturing hES cells with endothelial cells in the matrigel for separation of the mixed population of cells for transplantation therapy.

Thus, the claimed invention as a whole, is clearly prima facie obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Magdalene K. Sgagias whose telephone number is (571) 272-3305. The examiner can normally be reached on Monday through Friday from 9:00 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, Jr., can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR

system, see http://pair-direct.uspto.gov. Should you have questions on access to private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

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